	L #	Hits	Search Text	DBs	Time Stamp
1	L1	722	chitinase$1 or chitotriosidase$1	USPAT	2000/11/16 08:13
2	L2	6	1 near4 human	USPAT	2000/11/16 08:14

	Document ID	Issue Date	Pages	Title
1	US 6090573 A	20000718	43	Detecting eubacteria and fungus and determining their antibiotic sensitivity by using catalytically inactive murein binding enzymes
2	US 6057142 A	20000502	25	Human chitinase, its recombinant production, its use for decomposing chitin, its use in therapy or prophylaxis against infection diseases
3	US 5935804 A	19990810	43	Method for detecting eubacteria in biological samples with catalytically inactive murein binding enzymes
4	US 5928928 A	19990727	25	Human chitinase, its recombinant production, its use for decomposing chitin, its use in therapy or prophylaxis against infection diseases
5	US 5843449 A	19981201	19	Proteins and novel peptides derived from autoantigen for use in immunotherapy of autoimmune diseases
6	US 5811535 A	19980922	32	Human cartilege gp39-like gene

USPT

US-CL-CURRENT: 435/18,435/206 ,435/29 ,435/34

US-PAT-NO: 6090573
DOCUMENT-IDENTIFIER: US 6090573 A
TITLE: Detecting eubacteria and fungus and determining their antibiotic sensitivity by using catalytically inactive murein binding enzymes
DATE-ISSUED: July 18, 2000
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Laine; Roger A.	Baton Rouge	LA	70808	N/A
Lo; Wai Chun Jennifer	Baton Rouge	LA	70808	N/A

US-CL-CURRENT: 435/32,435/18 ,435/206 ,435/29 ,435/34
ABSTRACT:

A method is presented for detecting eubacteria and fungus in biological samples, and, if present, determining their antibiotic sensitivity, where the presence and amount of eubacteria or fungus is measured by means of catalytically inactive murein binding enzymes in both parts of the method. The sensitivity of eubacteria or fungus to a test antibiotic is obtained by comparing the amount of eubacteria or fungus in the sample after culturing the sample in the presence and absence of the test antibiotic. The biological sample may be chemically treated with alkali to cleave peptide bonds in the sample before incubating the sample with the catalytically inactive murein binding enzyme.

34 Claims, 31 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 11

DEPU:
7: Escott, G. M. and Adams, D. J. 1995. Chitinase activity in human serum and leukocytes. Inf. Imm. 63: 4770-4773.

USPT

US-CL-CURRENT: 435/252.3,435/320.1 ,435/325 ,536/23.2

US-PAT-NO: 6057142
DOCUMENT-IDENTIFIER: US 6057142 A
TITLE: Human chitinase, its recombinant production, its use for decomposing chitin, its use in therapy or prophylaxis against infection diseases
DATE-ISSUED: May 2, 2000
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Aerts; Johannes Maria Franciscus Gerardus	Abcoude	N/A	N/A	NLX

US-CL-CURRENT: 435/209,435/252.3 ,435/320.1 ,435/325 ,536/23.2
ABSTRACT:
A new human chitinase having an amino acid sequence as shown in FIG. 1 or FIG. 2. Modified forms of it having a similar chitin-hydrolyzing activity, and antigenic peptides representing one of its epitopes. Recombinant production of the human chitinase by genetically engineered hosts or host cells. Recombinant nucleic acid encoding it, and human chitinase-specific oligonucleotides. Use for therapeutic or prophylactic treatment of humans against infection by chitin-containing pathogens, or for decomposing chitin, e.g. from chitin-based articles. Antibodies binding to the human chitinase. Diagnostic test kits comprising the human chitinase, its antigenic peptides, human chitinase antibodies, recombinant nucleic acid or oligonucleotides.
6 Claims, 5 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 5

TTL:
Human chitinase, its recombinant production, its use for decomposing chitin, its use in therapy or prophylaxis against infection diseases

ABPL:
A new human chitinase having an amino acid sequence as shown in FIG. 1 or FIG. 2. Modified forms of it having a similar chitin-hydrolyzing activity, and antigenic peptides representing one of its epitopes. Recombinant production of the human chitinase by genetically engineered hosts or host cells. Recombinant nucleic acid encoding it, and human chitinase-specific oligonucleotides. Use for therapeutic or prophylactic treatment of humans against infection by chitin-containing pathogens, or for decomposing chitin, e.g. from chitin-based articles. Antibodies binding to the human chitinase. Diagnostic test kits comprising the human chitinase, its antigenic peptides, human chitinase antibodies, recombinant nucleic acid or oligonucleotides.

BSPR:
Secondly, the use of chitinases from pathogenic organisms as a vaccine may result in unforeseen harmful side-effects. It cannot be excluded that fragments of such chitinases share homology with endogenous proteins and that an undesired immune response is elicited. This may in fact be more than a theoretical problem because of the strong homology between human chitinase and chitinases from other species (see below).

BSPR:
Given the limitations of current approaches to tackle chitin-containing pathogens, a novel approach is here proposed to solve the problem that constitutes a major threat to the welfare of man. The approach is based on the use of a recently identified human chitinase, which can be produced by recombinant DNA technology (biotechnology), as a safe and effective agent against chitin-containing pathogens, i.e. for intervention of infectious diseases caused by chitin-containing pathogens. The conception of the approach and its further development is described below.

BSPR:
The subject invention provides a substantially isolated or purified chitinase,

said chitinase being a human chitinase having an amino acid sequence essentially corresponding to the amino acid sequence shown in FIG. 1 (SEQ ID NO:4) or the amino acid sequence shown in FIG. 2, (SEQ ID NO:6) or being a modified form of said human chitinase having a substantially similar chitin-hydrolyzing activity. It is preferred that this new human chitinase is produced by a genetically engineered host cell and isolated from said host cell or medium in which said host cell is cultured, wherein the amino acid sequence of the enzyme is encoded by a nucleotide sequence essentially corresponding to the nucleotide sequence shown in FIG. 1 (SEQ ID NO:3) or the nucleotide sequence shown in FIG. 2 (SEQ ID NO: 5). The subject invention particularly includes a chitinase having an amino acid sequence essentially corresponding to the amino acid sequence shown in FIG. 1 (SEQ ID NO:4) and having a molecular weight of about 50 kDa, and a chitinase having an amino acid sequence essentially corresponding to the amino acid sequence shown in FIG. 2 (SEQ ID NO:6) and having a molecular weight of about 39 kDa.

BSPR:
The phrase "essentially corresponding to" intends to allow for small sequence variations, such as the naturally occurring variations which do not significantly affect the activity of the enzyme. Some amino acids of the human chitinase sequence may be replaced by others, or be deleted, without thereby significantly affecting the function, activity and tolerability of the enzyme, and may sometimes even improve one characteristic or the overall properties of the enzyme. Generally, such sequence variations will be quite limited, say to about less than 30%, more often less than 20% or even less than 10% of all amino acids, i.e. the variants will generally have a high homology of above 70%, more often above 80% or even above 90%, compared to the sequences shown in FIGS. 1 and 2 (SEQ ID NOS:4 and 6). All have in common the functional characteristic of chitinase activity, which can be measured for typical chitinase substrates, such as 4-methylumbelliferyl-chitotrioside.

BSPR:
The phrase "a modified form of said human chitinase having a substantially similar chitin-hydrolyzing activity" intends to embrace variants whose amino acid sequence differs significantly from the sequences shown in FIGS. 1 and 2 (SEQ ID NOS:4 and 6) but which yet have a similar chitinase activity. Such modified forms having similar or even improved properties could be designed on the basis of the module or domain structure of the human chitinase, such as constructs lacking a domain which is not required or even disadvantageous for activity, and constructs containing two or more copies of a domain whose amplified presence is desirable.

BSPR:
The phrase "having a substantially similar chitin-hydrolyzing activity" intends to set the minimum requirement of having an at least equivalent chitinase activity compared to the human chitinases shown in FIGS. 1 and 2 (SEQ ID NOS:3-6). "Equivalent" refers to equivalency in substrate range, i.e. qualitatively, and to equivalency in activity value, i.e. quantitatively.

BSPR:
The subject invention furthermore provides a pharmaceutical composition comprising the new human chitinase as defined herein and a

BSPR:
pharmaceutically acceptable carrier or diluent, more in particular a pharmaceutical composition for therapeutic or prophylactic treatment of a human individual against infection by a chitin-containing pathogen, comprising a therapeutically or prophylactically effective amount of the new human chitinase and a pharmaceutically acceptable carrier or diluent. Preferably the pharmaceutical composition further comprises a therapeutically or prophylactically effective amount of a human .beta.-1,3-glucanase.

BSPR:
The invention also provides non-pharmaceutical compositions comprising the new human chitinase and a carrier or diluent. For example, such composition may be a medium for culturing cells, in particular human cells, or be a cosmetic (e.g. body lotion), dental (e.g. tooth paste, mouth rinse) or food product (e.g. milk, cheese and other dairy products).

BSPR:
Furthermore, this invention provides chitin-based articles of manufacture comprising a chitin-hydrolyzing amount of the new human chitinase. E.g., the chitin-based article of manufacture may be a drug-containing drug carrier or implant for controlled drug release, or a transient functional implant.

BSPR:
This invention also provides a method of therapeutic or prophylactic treatment of a human individual against infection by a chitin-containing pathogen, comprising administering to said human individual a pharmaceutical composition comprising a therapeutically or prophylactically effective amount of the new human chitinase.

BSPR:
The subject invention also provides a process for preparing a human chitinase having an amino acid sequence essentially corresponding to the amino acid sequence shown in FIG. 1 (SEQ ID NO:4) or the amino acid sequence shown in FIG. 2 (SEQ ID NO:6), or a modified form of said human chitinase having a substantially similar chitin-hydrolyzing activity, comprising growing a genetically engineered host or host cell capable of producing said human chitinase or modified form thereof and isolating the chitinase produced from said host or host cell or from medium in which said host cell is cultured. In this process, preferably the amino acid sequence of said chitinase is encoded by a nucleotide sequence essentially corresponding to the nucleotide sequence shown in FIG. 1 (SEQ ID NO:3) or the nucleotide sequence shown in FIG. 2 (SEQ ID NO:5).

BSPR:
The invention also provides a genetically engineered host cell capable of producing a human chitinase having an amino acid sequence essentially corresponding to the amino acid sequence shown in FIG. 1 (SEQ ID NO:4) or the amino acid sequence shown in FIG. 2 (SEQ ID NO:6), or a modified form of said human chitinase having a substantially similar chitin-hydrolyzing activity.

BSPR:
The invention also embraces oligonucleotides of at least about 8 nucleotides having a nucleotide sequence corresponding to, or complementary to, a nucleotide sequence shown in FIG. 1 (SEQ ID NO:3) or a nucleotide sequence shown in FIG. 2 (SEQ ID NO:5) and being capable of binding by hybridisation under stringent (i.e. requiring about complete complementarity) hybridisation conditions to nucleic acid coding for the new human chitinase. Such oligonucleotides can be useful for different purposes, e.g. as a primer for use in nucleic acid amplification methods such as PCR, NASBA etc., or as a probe in hybridisation analysis. The length will usually depend on the intended use. When used as a primer, the length will normally be between 12, preferably 15, and 25, preferably 20 nucleotides. When used as a probe, the length will usually be somewhat higher, e.g. from about 15 or 20 up to about 40 or 50 nucleotides, or even up to the complete length of the coding sequence.

BSPR:
Similarly, this invention furthermore embraces peptides of at least about 8 amino acid residues having an amino acid sequence derived from the amino acid sequence shown in FIG. 1 (SEQ ID NO:4) or the amino acid sequence shown in FIG. 2 (SEQ ID NO:6) and representing or mimicking an epitope of the new human chitinase, in particular those having an amino acid sequence corresponding to an amino acid sequence shown in FIG. 1 (SEQ ID NO:4) or an amino acid sequence shown in FIG. 2 (SEQ ID NO:6) and having antigenicity. Usually, such peptides will have a length of at least about 10, or even at least about 15 amino acid residues, and up to about 40, preferably up to about 30 amino acid residues. Said peptides can be used for diagnostic purposes, or in immunization protocols to raise human chitinase-specific antibodies.

BSPR:
The invention also embraces antibodies capable of binding to the new human chitinase, especially monoclonal antibodies. Such antibodies can be used for many purposes, for example for isolating and/or purifying (e.g. by affinity chromatography) the human chitinase, or for diagnostic purposes.

BSPR:
The subject invention furthermore provides a diagnostic kit comprising such a human chitinase-binding antibody, or a human chitinase peptide as defined above, or the new human chitinase itself as defined herein, together with a conventional component of diagnostic kits for detecting an antigen or an antibody; and a diagnostic kit comprising a human chitinase-specific oligonucleotide or recombinant human chitinase-encoding nucleic acid as defined herein, together with a conventional component of diagnostic kits for detecting a nucleic acid.

BSPR:
Furthermore, the subject invention provides a method of decomposing chitin comprising contacting said chitin with the new human chitinase under chitin-hydrolyzing conditions.

DEPR:
The fact that man is continuously exposed to chitin (or chitin-containing organisms) strongly suggests that man should also have the ability to degrade this material. A gradual, presumably lysosomal, accumulation of chitin would otherwise inevitably occur in life, e.g. in alveolar macrophages that are in continuous contact with chitin-containing organisms. However, such storage of chitin has never been noted. This prompted us to search for the occurrence of a chitinase activity in human macrophages. Indeed, as is documented below, we were able to demonstrate that, in contrast to previous believes, macrophages can produce a chitinase similar in properties to enzyme encountered in other non-mammalian organisms (17,18). The enzyme is highly capable of hydrolyzing chitin and also shows other common characteristics of chitinases. Based on the substrate initially used in the identification of the novel enzyme, i.e. 4-methylumbelliferyl-chitotrioside, the human chitinase has been named chitotriosidase (17).

DEPR:
In order to use the human chitinase (chitotriosidase) as a (pharmaceutical) agent against chitin-containing organisms in vivo, a number of conditions have to be fulfilled.

DEPR:
In order to be useful as an agent against chitin-containing pathogens chitotriosidase has furthermore to be available in large quantities in a uniform state. There are no ubiquitous, natural sources for the isolation of the human chitinase. The mounts of enzyme in urine and placentas are low. This led us to attempt to isolate cDNA encoding chitotriosidase. Due to the specific expression of the chitotriosidase gene in macrophages, all tested cDNA libraries from other cell types were found to be negative for chitotriosidase cDNA. However, a constructed cDNA library from mRNA of long-term cultured macrophages that secreted massive amounts of chitotriosidase activity proved to be extremely rich in cDNAs encoding chitotriosidase, (0.1% of total CDNA). Two distinct cDNAs were in this manner identified and cloned.

DEPR:
The findings suggest that large scale recombinant production of both forms of human chitotriosidase using conventional techniques should be feasible. Moreover, it seems likely that not only production of the human enzyme in eukaryotic cells, but even in prokaryotes might be possible, since highly homologous proteins are endogenously produced by some of these organisms, e.g. Serratia marcescens. A procedure for the purification of chitotriosidase has been successfully developed (18; and below) It therefore will be possible to obtain large amounts of both recombinant

DEPR:
human chitotriosidases in a pure and uniform state suitable for administration to man.

DEPR:
The 39 kDa chitozyme is not a glycosylated protein, so its production in prokaryotic cells should certainly be feasible. Bacteria which produce and secrete highly homologous chitinases should in principle be able to secrete

correctly folded human chitotriosidase in their exoplasmic space, provided that a correct leader sequence is used. Alternatively, it could be considered to use yeast cells for the production of recombinant chitotriosidase, at least the 39 kDa chitozyme. It can so far not be excluded, however, that also 50 kDa chitotriosidase can be produced, not only in higher eukaryotes, but also in lower eukaryotes or even in prokaryotes.

DEPR:
A prerequisite for the intravenous application of the chitinase is insight in its clearance. In the blood stream the most predominant isozyme is the 50 kDa protein. In tissue predominantly a 39 kDa isozyme is encountered. This appears to be formed by uptake of 50 kDa protein followed by proteolytic cleavage to a 39 kDa form that is remarkably stable in the lysosomal environment. Experiments in rats suggest that the half life of recombinant 50 kDa chitotriosidase in the circulation is somewhat longer than that of the 39 kDa enzyme. Clearance is not a very rapid process as monitored by the disappearance of activity of human chitotriosidase in the blood stream of intravenously injected rats, the half life being about one hour. Only minor amounts of chitotriosidase are daily excreted into the urine. It is conceivable that some enzyme is efficiently recaptured by proximal tube epithelial cells since kidney is found to be extremely rich in `lysosomally processed` 39 kDa enzyme. The observations so far suggest that intravenous administration can lead to a high level of human chitinase activity in the circulation for a prolonged period of time, allowing enzyme to reach various tissue locations.

DEPR:
A. Use of a cocktail of recombinant human chitinase and .beta.-1,3-glucanase

DEPR:
It is well documented that both in plants and fish chitinases play an important role in resistance against fungal infections. In plants, chitinases act synergistically with .beta.-1,3 glucanases since the cell walls of fungi are composed of a mixture of chitin and .beta.-glucan fibrils (15). At present it is believed that man is not capable of producing a chitinase nor a .beta.-glucanase. However, it was noted that long-term cultured macrophages are not only able to secrete a chitinolytic enzyme but also an enzyme active against dye-labeled .beta.-glucan. We therefore propose that analogous to the situation in plants a mixture of human chitinase and .beta.-glucanase could be a more powerful anti-fungal agent than one of these enzymes alone. Isolation of the .beta.-glucanase produced by long-term cultured macrophages and subsequent cloning of corresponding cDNA, should result in the availability of recombinant human .beta.-glucanase for this purpose.

DEPR:
B. Use of modified recombinant human chitinase

DEPR:
It cannot be excluded that a deficiency in chitotriosidase may be associated with some disadvantage. For example, the resistance against chitin-containing pathogens could be reduced and lysosomal degradation of chitin in phagocytes could be impaired, resulting in abnormal behaviour of the cells. Further research is required to establish whether a chitotriosidase deficiency is indeed associated with some risks. If this proofs to be the case, prophylactic administration of human chitotriosidase to deficient individuals could be considered.

DEPR:
The availability of a human chitinase could be also exploited as a tool to degrade injected or implanted chitin-based structures for medical purposes.

DEPR:
For example, drugs could be incorporated in chitin based capsules (`chitosomes`). The concomitant presence of well defined amounts of human chitinase in the capsule could ensure a controlled release of drugs. A slow but gradual release of drug could particularly be envisioned when it is trapped in a chitin matrix. The use of the human enzyme in such a system would result in ultimate destruction of the chitin-based capsule and not elicit an

immunological response. The drugs used in such a system could vary from small compounds to proteins and DNA fragments for the purpose of enzyme and gene therapy. Chitin (or analogues) is already employed as a carrier for drugs (20).

DEPR:
Finally, recombinant human chitotriosidase (or a cocktail with .beta.-1,3-glucanase) may be used as an additive in tooth paste and body lotions in order to prevent fungal infections.

DEPR:
In order to clone cDNA encoding human chitotriosidase the following strategy was used. Chitotriosidase was purified from spleen of a type 1 Gaucher disease patient since this organ is extremely rich in chitotriosidase activity (18). The N-terminal amino acid sequence of chitotriosidase was determined and this information was used for cloning chitotriosidase cDNA. Firstly, the established N-terminal amino acid sequence of chitotriosidase (18) was used to design a degenerate sense oligonucleotide: 5'-TGYTAYTTYACNAAYTGGGC-3'(SEQ ID NO:1). Secondly, a degenerate anti-sense nucleotide was designed based on the highly conserved domain among chitinases that is presumed to be an essential part of the catalytic center: 5'-CCARTCIARRTYIACICCRTCRAA-3'(SEQ ID NO:2).

DEPR:
These oligonucleotides were used to amplify a DNA fragment by RT-PCR. For this purpose, total RNA had been isolated from long-term cultured macrophages that secreted large amounts of chitotriosidase activity. First strand cDNA synthesis was performed using SuperScript TM RNAse H, reverse transcriptase and oligo dT. After alkaline hydrolysis, the cDNA was precipitated with ethanol and used as template. PCR was performed using standard conditions. The DNA fragment obtained by RT-PCR was of the expected size (on the basis of homology with members of the chitinase family). The fragment was purified, treated with T4 DNA polymerase and cloned into the HindII site of the plasmid vector pUC19. Determination of its sequence using the dideoxynucleotide chain termination method revealed that the fragment was in complete accordance with the known N-terminal amino acid sequence of purified human chitotriosidase, allowing its use as a probe to identify a full length chitotriosidase cDNA.

DEPR:
followed by the N-terminal sequence established for the chitotriosidase protein. The cDNA sequence does not indicate the presence of potential N-linked glycosylation sites, which is consistent with the absence of N-linked glycans in isolated chitotriosidase. The predicted protein, after removal of the signal sequence, has a length of 445 amino acids and a calculated molecular mass of 49 kDa. Metabolic labelling experiments with cultured macrophages revealed that these cells predominantly synthesize and secrete a chitotriosidase protein with apparent molecular mass of 50 kDa with polyacrylamide gel electrophoresis in the presence of sodium dodecylsulphate at reducing conditions. The predicted C-terminal part of 50 kDa human chitotriosidase is rich in serine residues of which theoretically some might be O-linked glycosylated. The occurrence of this type of glycans in 50 kDa human chitotriosidase has so far not been excluded or confirmed.

DEPR:
A search of the EMBL and GenBank databases revealed significant homology between the two human chitotriosidases and a group of chitinases and related proteins from different species. All the homologous proteins belong to the so called `chitinase protein family` (18,19).

DEPR:
The predicted C-terminal part of 50 kDa human chitotriosidase shows only homology with two chitinases from Manduca sexta and Brugia malayi, respectively. In the case of the latter enzyme O-linked glycosylation has been reported (12).

DEPR:
The chitotriosidase produced by COS cells was analysed by immunotitration with a rabbit antiserum against human chitotriosidase. This antiserum is capable of inhibiting human chitotriosidase in its enzymatic activity. FIG. 5 shows that

chitotriosidase is inactivated by the antiserum in an identical manner to isolated splenic chitotriosidase. This finding suggests that the enzymatic activity per amount of antigen is similar in the case of the two recombinant chitotriosidases and the splenic enzyme.

DEPR:
Chitin azure (Sigma) suspended in citrate/phosphate buffer (pH 5.2) at a final concentration of 10 mg/ml was used to monitor chitinase activity. Chitin degradation was detected spectrophotometrically at 550 nm by determination of release of soluble azure (18). Chitinase from Serratia marcescens (Sigma) was used as control. When related to the hydrolysis of 4-methylumbelliferyl-chitotrioside, the chitinase activity of human chitotriosidase was comparable to that of the bacterial chitinase. See for example ref.18.

DEPR:
No significant activity of human chitotriosidase towards a cell wall suspension of Micrococcus lysodeikticus was detectable, suggesting that the enzymes lack lysozyme activity.

DEPR:
To test whether human chitotriosidase can exert an anti-fungal action, a chitinous fungus (Mucor mucedo) was grown on plates (containing malt extract, peptone, glucose and agar) under a Cellophane membrane in order to keep the hyphae flat against the agar surface (see ref.16). Individual sectors were cut out and mounted on microscope slides. Purified chitozyme 50 and chitozyme 39 were dialysed against 0.15 M sodium chloride. Samples of enzyme-containing solutions, and 0.15 M NaCl were pipetted on the hyphal tips. Microscopical analysis revealed that application of enzyme resulted in immediate cessation of hyphal growth, followed by a distorted morphological appearance. Application of saline had no effect. Negative effects on hyphal growth were detectable using chitozyme solutions with a concentration of enzyme as little as 0.005 mg/ml.

DEPR:
The proteins are: human chitotriosidase; (SEQ ID NO:7) a chitinase from the virus Autographa californica (GenBank L22858); (SEQ ID NO:8) a chitinase from the tobacco hornworm Manduca sexta (GenBank U02270) (SEQ ID NO:7); an endochitinase from the nematode Brugia malayi (Genbank M73689) (SEQ ID NO:9); a human oviductal glycoprotein (GenBank U09550) (SEQ ID NO:10); HCgp-39, a human glycoprotein produced by chondrocytes and synovial cells (GenBank M80927); (SEQ ID NO:11) YM-1, a secretory protein of activated mouse macrophages (Pir S27879) (SEQ ID NO:12); a chitinase from the fungus Aphanocladium album (SwissProt P32470) (SEQ ID NO:13); a chitinase from the filamentous fungus Trichoderma harzianum (GenBank L14614); (SEQ ID NO:14) chitinase A1 from the prokaryote Bacillus circulans (SwissProt P20533) (SEQ ID NO:15); and a class V chitinase from the plant Nicotiana tabacum (GenBank X77110) (SEQ ID NO:16). Residues identical to chitotriosidase are indicated by the inverted characters. The proteins HCgp-39 and YM-1 are supposed to be not chitinolytic.

DEPR:
Preparations containing either purified 39 kDa splenic chitotriosidase (), or 50 kDa chitozyme produced by COS cells transfected with chi.50 cDNA (), or 39 kDa chitozyme produced by COS cells transfected with chi.39 cDNA were incubated for 1 hour at room temperature in phosphate buffered saline with different amounts of rabbit (anti-human splenic chitotriosidase) antiserum.

DEPL:
Cloning and composition of cDNAs encoding human chitotriosidases

DEPL:
Recombinant production of human chitotriosidases

DEPU:
18. Renkema, G. H., Boot, R. G., Muijsers, A. O., Donker-Koopman, W. E., Aerts, J. M. F. G. (1995), J. Biol. Chem. 270, 2198-2202. Purification and characterization of human chitotriosidase, a novel member of the chitinase family of proteins.

CLPR:
1. A recombinant host cell comprising a nucleic acid having a nucleotide sequence encoding the human chitinase of SEQ ID NO:4 or SEQ ID NO:6 or a nucleotide sequence encoding a chitinase with having substantially similar chitin-hydrolyzing activity to the human chitinase of SEQ ID NO:4 or SEQ ID NO:6, wherein said nucleic acid will hybridize to a nucleic acid of SEQ ID NO:3 or SEQ ID NO:5 at 65.degree. C. in 1 mM EDTA, 0.5M sodium hydrogen phosphate buffer (pH7.2) containing 7% (w/v) SDS.

CLPR:
2. The recombinant host cell of claim 1, wherein said nucleotide sequence encodes the human chitinase of SEQ ID NO:4 or SEQ ID NO:6.

CLPR:
5. The process of producing a chitinase of claim 4, wherein said nucleotide sequence encodes the human chitinase of SEQ ID NO:4 or SEQ ID NO:6.

USPT

US-CL-CURRENT: 435/206,435/29

US-PAT-NO: 5935804
DOCUMENT-IDENTIFIER: US 5935804 A
TITLE: Method for detecting eubacteria in biological samples with catalytically inactive murein binding enzymes
DATE-ISSUED: August 10, 1999
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Laine; Roger A.	Baton Rouge	LA	70808	N/A
Lo; Wai C. J.	Baton Rouge	LA	70808	N/A

US-CL-CURRENT: 435/18,435/206 ,435/29
ABSTRACT:
A method for detecting eubacteria in biological samples with catalytically inactive murein binding enzyme is presented. The biological sample may be chemically treated with alkali to cleave peptide bonds in the sample before incubating the sample with the catalytically inactive murein binding enzyme.
23 Claims, 31 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 11

DEPR:
7. Escott G. M. and Adams, D. L. 1995. Chitinase activity in human serum and leukocytes. Inf Imm. 63: 4770-4773.

USPT

US-CL-CURRENT: 435/183,530/350 ,536/23.1 ,536/24.3

US-PAT-NO: 5928928
DOCUMENT-IDENTIFIER: US 5928928 A
TITLE: Human chitinase, its recombinant production, its use for decomposing chitin, its use in therapy or prophylaxis against infection diseases
DATE-ISSUED: July 27, 1999
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Aerts; Johannes Maria Franciscus Gerardus	Abcoude	N/A	N/A	NLX

US-CL-CURRENT: 435/201,435/183 ,530/350 ,536/23.1 ,536/24.3
ABSTRACT:
A new human chitinase having an amino acid sequence as shown in FIG. 1 or FIG. 2. Modified forms of it having a similar chitin-hydrolyzing activity, and antigenic peptides representing one of its epitopes. Recombinant production of the human chitinase by genetically engineered hosts or host cells. Recombinant nucleic acid encoding it, and human chitinase-specific oligonucleotides. Use for therapeutic or prophylactic treatment of humans against infection by chitin-containing pathogens, or for decomposing chitin, e.g. from chitin-based articles. Antibodies binding to the human chitinase. Diagnostic test kits comprising the human chitanase, its antigenic peptides, human chitinase antibodies, recombinant nucleic acid or oligonucleotides.
24 Claims, 5 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 5

TTL:
Human chitinase, its recombinant production, its use for decomposing chitin, its use in therapy or prophylaxis against infection diseases

ABPL:
A new human chitinase having an amino acid sequence as shown in FIG. 1 or FIG. 2. Modified forms of it having a similar chitin-hydrolyzing activity, and antigenic peptides representing one of its epitopes. Recombinant production of the human chitinase by genetically engineered hosts or host cells. Recombinant nucleic acid encoding it, and human chitinase-specific oligonucleotides. Use for therapeutic or prophylactic treatment of humans against infection by chitin-containing pathogens, or for decomposing chitin, e.g. from chitin-based articles. Antibodies binding to the human chitinase. Diagnostic test kits comprising the human chitanase, its antigenic peptides, human chitinase antibodies, recombinant nucleic acid or oligonucleotides.

BSPR:
Secondly, the use of chitinases from pathogenic organisms as a vaccine may result in unforeseen harmful side effects. It cannot be excluded that fragments of such chitinases share homology with endogenous proteins and that an undesired immune response is elicited. This may in fact be more than a theoretical problem because of the strong homology between human chitinase and chitinases from other species (see below).

BSPR:
Given the limitations of current approaches to tackle chitin-containing pathogens, a novel approach is here proposed to solve the problem that constitutes a major threat to the welfare of man. The approach is based on the use of a recently identified human chitinase, which can be produced by recombinant DNA technology (biotechnology), as a safe and effective agent against chitin-containing pathogens, i.e. for intervention of infectious diseases caused by chitin-containing pathogens. The conception of the approach and its further development is described below.

BSPR:
The subject invention provides a substantially isolated or purified chitinase,

said chitinase being a human chitinase having an amino acid sequence essentially corresponding to the amino acid sequence shown in FIG. 1 or the amino acid sequence shown in FIG. 2, or being a modified form of said human chitinase having a substantially similar chitin-hydrolyzing activity. It is preferred that this new human chitinase is produced by a genetically engineered host cell and isolated from said host cell or medium in which said host cell is cultured, wherein the amino acid sequence of the enzyme is encoded by a nucleotide sequence essentially corresponding to the nucleotide sequence shown in FIG. 1 or the nucleotide sequence shown in FIG. 2. The subject invention particularly includes a chitinase having an amino acid sequence essentially corresponding to the amino acid sequence shown in FIG. 1 and having a molecular weight of about 50 kDa, and a chitinase having an amino acid sequence essentially corresponding to the amino acid sequence shown in FIG. 2 and having a molecular weight of about 39 kDa.

BSPR:
The phrase "essentially corresponding to" intends to allow for small sequence variations, such as the naturally occurring variations which do not significantly affect the activity of the enzyme. Some amino acids of the human chitinase sequence may be replaced by others, or be deleted, without thereby significantly affecting the function, activity and tolerability of the enzyme, and may sometimes even improve one characteristic or the overall properties of the enzyme. Generally, such sequence variations will be quite limited, say to about less than 30%, more often less than 20% or even less than 10% of all amino acids, i.e. the variants will generally have a high homology of above 70%, more often above 80% or even above 90%, compared to the sequenase shown in FIGS. 1 and 2. All have in common the functional characteristic of chitinase activity, which can be measured for typical chitinase substrates, such as 4-methylumbelliferyl-chitotrioside.

BSPR:
The phrase "a modified form of said human chitinase having a substantially similar chitin hydrolyzing activity" intends to embrace variants whose amino acid sequence differs significantly from the sequences shown in FIGS. 1 and 2 but which yet have a similar chitinase activity. Such modified forms having similar or even improved properties could be designed on the basis of the module or domain structure of the human chitinase, such as constructs lacking a domain which is not required or even disadvantageous for activity, and constructs containing two or more copies of a domain whose amplified presence is desirable.

BSPR:
The phrase "having a substantially similar chitin-hydrolyzing activity" intends to set the minimum requirement of having an at least equivalent chitinase activity compared to the human chitinases shown in FIGS. 1 and 2. "Equivalent" refers to equivalency in substrate range, i.e. qualitatively, and to equivalency in activity value, i.e. quantitatively.

BSPR:
The subject invention furthermore provides a pharmaceutical composition comprising the new human chitinase as defined herein and a pharmaceutically acceptable carrier or diluent, more in particular a pharmaceutical composition for therapeutic or prophylactic treatment of a human individual against infection by a chitin-containing pathogen, comprising a therapeutically or prophylactically effective amount of the new human chitinase and a pharmaceutically acceptable carrier or diluent. Preferably the pharmaceutical composition further comprises a therapeutically or prophylactically effective amount of a human .beta.-1,3-glucanase.

BSPR:
The invention also provides non-pharmaceutical compositions comprising the new human chitinase and a carrier or diluent. For example, such composition may be a medium for culturing cells, in particular human cells, or be a cosmetic (e.g. body lotion), dental (e.g. tooth paste, mouth rinse) or food product (e.g. milk, cheese and other dairy products).

BSPR:
Furthermore, this invention provides chitin-based articles of manufacture

comprising a chitin-hydrolyzing amount of the new human chitinase. E.g., the chitin-based article of manufacture may be drug-containing drug carrier or implant for controlled drug release, or a transient functional implant.

BSPR:
This invention also provides a method of therapeutic or prophylactic treatment of a human individual against infection by a chitin-containing pathogen, comprising administering to said human individual a pharmaceutical composition comprising a therapeutically or prophylactically effective amount of the new human chitinase.

BSPR:
The subject invention also provides a process for preparing a human chitinase having an amino acid sequence essentially corresponding to the amino acid sequence shown in FIG. 1 or the amino acid sequence shown in FIG. 2, or a modified form of said human chitinase having a substantially similar chitin-hydrolyzing activity, comprising growing a genetically engineered host or host cell capable of producing said human chitinase or modified form thereof and isolating the chitinase produced from said host or host cell or form medium in which said host cell is cultured. In this process, preferably the amino acid sequence of said chitinase is encoded by a nucleotide sequence essentially corresponding to the nucleotide sequence shown in FIG. 1 or the nucleotide sequence shown in FIG. 2.

BSPR:
The invention also provides a genetically engineered host cell capable of producing a human chitinase having an amino acid sequence essentially corresponding to the amino acid sequence shown in FIG. 1 or the amino acid sequence shown in FIG. 2, or a modified form of said human chitinase having a substantially similar chitin-hydrolyzing activity.

BSPR:
The invention also embraces oligonucleotides of at least about 8 nucleotides having a nucleotide sequence corresponding to, or complementary to, a nucleotide sequence shown in FIG. 1 or a nucleotide sequence shown in FIG. 2 and being capable of binding by hybridisation under stringent (i.e. requiring about complete complementarity) hybridisation conditions to nucleic acid coding for the new human chitinase. Such oligonuclotides can be useful for different purposes, e.g. as a primer for use in nucleic acid amplification methods such as PCR, NASBA etc., or as a probe in hybridisation analysis. The length will usually depend on the intended use. When used as a primer, the length will normally be between 12, preferably 15, and 25, preferably 20 nucleotides. When used as a probe, the length will usually be somewhat higher, e.g. from about 15 or 20 up to about 40 or 50 nucleotides, or even up to the complete length of the coding sequence.

BSPR:
Similarly, this invention furthermore embraces peptides of at least about 8 amino acid residues having an amino acid sequence derived from the amino acid sequence shown in FIG. 1 or the amino acid sequence shown in FIG. 2 and representing or mimicking an epitope of the new human chitinase, in particular those having an amino acid sequence corresponding to an amino acid sequence shown in FIG. 1 or an amino acid sequence shown in FIG. 2 and having antigenicity. Usually, such peptides will have a length of at least about 10, or even at least about 15 amino acid residues, and up to about 40, preferably up to about 30 amino acid residues. Said peptides can be used for diagnostic purposes, or in immunization protocols to raise human chitinase-specific antibodies.

BSPR:
The invention also embraces antibodies capable of binding to the new human chitinase, especially monoclonal antibodies. Such antibodies can be used for many purposes, for example for isolating and/or purifying (e.g. by affinity chromatography) the human chitinase, or for diagnostic purposes.

BSPR:
The subject invention furthermore provides a diagnostic kit comprising such a human chitinase-binding antibody, or a human chitinase peptide as defined

above, of the new **human chitinase** itself so defined herein, together with a conventional component of diagnostic kits for detecting an antigen or an antibody; and a diagnostic kit comprising a **human chitinase-specific oligonucleotide or recombinant human chitinase**-encoding nucleic acid as defined herein, together with a conventional component of diagnostic kits for detecting a nucleic acid.

BSPR:
Furthermore, the subject invention provides a method of decomposing chitin comprising contacting said chitin with the new **human chitinase** under chitin-hydrolyzing conditions.

DEPR:
The fact that man is continuously exposed to chitin (or chitin-containing organisms) strongly suggests that man should also have the ability to degrade this material. A gradual, presumably lysosomal, accumulation of chitin would otherwise inevitably occur in life, e.g. in alveolar macrophages that are in continuous contact with chitin-containing organisms. However, such storage of chitin has never been noted. This prompted us to search for the occurrence of a **chitinase activity in human** macrophages. Indeed, as is documented below, we were able to demonstrate that, in contrast to previous believes, macrophages can produce a chitinase similar in properties to enzyme encountered in other non-mammalian organisms (17,18). The enzyme is highly capable of hydrolyzing chitin and also shows other common characteristics of chitinases. Based on the substrate initially used in the identification of the novel enzyme, i.e. 4-methylumbelliferyl-chitotrioside, the **human chitinase** has been named chitotriosidase (17).

DEPR:
In order to use the **human chitinase** (chitotriosidase) as a (pharmaceutical) agent against chitin-containing organisms in vivo, a number of conditions have been fulfilled.

DEPR:
In order to be useful as an agent against chitin-containing pathogens chitotrioidase has furthermore to be available in large quantities in a uniform state. There are no ubiquitous, natural sources for the isolation of the **human chitinase**. The amounts of enzyme in urine and placentas are low. This led us to attempt to isolate cDNA encoding chitotriosidase. Due to the specific expression of the chitotriosidase gene in macrophages, all tested cDNA libraries from other cell types were found to be negative for chitotriosidase cDNA. However, a constructed cDNA library from mRNA of long-term cultured macrophages that secreted massive amounts of chitotriosidase activity proved to be extremely rich in cDNAs encoding chitotriosidase, (0.1% of total cDNA). Two distinct cDNAs were in this manner identified and cloned.

DEPR:
The findings suggest that large scale recombinant production of both forms of **human chitotriosidase** using conventional techniques should be feasible. Moreover, it seems likely that not only production of the human enzyme in eukaryotic cells, but even in prokaryotes might be possible, since highly homologous proteins are endogenously produced by some of these organisms, e.g. Serratia marcoscens. A procedure for the purification of chitotriosidase has been successfully developed (18; and below). It therefore will be possible to obtain large amounts of both recombinant **human chitotriosidases** in a pure and uniform state suitable for administration to man.

DEPR:
The 39 kDa chitozyme is not a glycosylated protein, so its production in prokaryotic cells should certainly be feasible. Bacteria which produce and secrete highly homologous chitinases should in principle be able to secrete correctly folded **human chitotriosidase** in their exoplasmic space, provided that a correct leader sequence is used. Alternatively, it could be considered to use yeast cells for the production of recombinant chitotriosidase, at least the 39 kDa chitozyme. It can so far not be excluded, however, that also 50 kDa chitotriosidase can be produced, not only in higher eukaryotes, but also in lower eukaryotes or even in prokaryotes.

DEPR:
A prerequisite for the intravenous application of the chitinase is insight in its clearance. In the blood stream the most predominant isozyme is the 50 kDa protein. In tissue predominantly a 39 kDa isozyme is encountered. This appears to be formed by uptake of 50 kDa protein followed by proteolytic cleavage to a 39 kDa form that is remarkably stable in the lysosomal environment. Experiments in rats suggest that the half life of recombinant 50 kDa chitotriosidase in the circulation is somewhat longer than that of the 39 kDa enzyme. Clearance is not a very rapid process as monitored by the disappearance of activity of human chitotriosidase in the blood stream of intra venously injected rats, the half life being about one hour. Only minor amounts of chitotriosidase are daily excreted into the urine. It is conceivable that some enzyme is efficiently recaptured by proximal tube epithelial cells since kidney is found to be extremely rich in `lysosomally processed` 39 kDa enzyme. The observations so far suggest that intravenous administration can lead to a high level of human chitinase activity in the circulation for a prolonged period of time, allowing enzyme to reach various tissue locations.

DEPR:
A. Use of a cocktail of recombinant human chitinase and .beta.-1,3-glucanase

DEPR:
It is well documented that both in plants and fish chitinases play an important role in resistance against fungal infections. In plants, chitinases act synergistically with .beta.-1,3-glucanases since the cell walls of fungi are composed of a mixture of chitin and .beta.-glucan fibrils (15). At present it is believed that man is not capable of producing a chitinase nor a .beta.-glucanase. However, it was noted that long-term cultured macrophages are not only able to secrete a chitinolytic enzyme but also an enzyme active against dye-labeled .beta.-glucan. We therefore propose that analogous to the situation in plants a mixture of human chitinase and .beta.-glucanase could be a more powerful anti fungel agent than one of these enzymes alone. Isolation of the .beta.-glucanase produced by long-term cultured macrophages and subsequent cloning of corresponding cDNA, should result in the availability of recombinant human .beta.-glucanase for this purpose.

DEPR:
B. Use or modified recombinant human chitinase

DEPR:
It cannot be excluded that a deficiency in chitotriosidase may be associated with some disadvantage. For example, the resistance against chitin-containing pathogens could be reduced and lysosomal degradation of chitin in phagocytes could be impaired, resulting in abnormal behaviour of the cells. Further research is required to establish whether a chitotriosidase deficiency is indeed associated with some risks. If these proofs to be the case, prophylactic administration of human chitotriosidase to deficient individuals could be considered.

DEPR:
The availability of a human chitinase could be also exploited as a tool to degrade injected or implanted chitin-based structures for medical purposes.

DEPR:
For example, drugs could be incorporated in chitin based capsules (`chitosomes`). The concomitant presence of well defined amounts of human chitinase in the capsule could ensure a controlled release of drugs. A slow but gradual release of drug could particularly be envisioned when it is trapped in a chitin matrix. The use of the human enzyme in such a system would result in ultimate destruction of the chitin-based capsule and not elicit an immunological response. The drugs used in such a system could vary from small compounds to proteins and DNA fragments for the purpose of enzyme and gene therapy. Chitin (or analogues) is already employed as a carrier for drugs (20).

DEPR:
Finally, recombinant human chitotriosidase (or a cocktail with .beta.-1,3-glucanase) may be used as an additive in tooth paste and body

lotions in order to prevent fungal infections.

DEPR:
In order to clone cDNA encoding human chitotriosidase the following strategy was used. Chitotriosidase was purified from spleen of a type 1 Gaucher disease patient since this organ is extremely rich in chitotriosidase activity (18). The N-terminal amino acid sequence of chitotriosidase was determined and this information was used for cloning chitotriosidase cDNA. Firstly, the established N-terminal amino acid sequence of chitotriosidase (18) was used to design a degenerate sense of oligonucleotide: 5'-TGYTAYTTYACNAAYTGGGC-3' (SEQ ID NO: 17). Secondary, a degenerate anti-sense nucleotide was designed based on the highly conserved domain among chitinases that is presumed to be an essential part of the catalytic center: 5'-CCARTCIARRTYIACICCRTCRAA 3' (SEQ ID NO: 18).

DEPR:
These oligonucleotides were used to amplify a DNA fragment by RT-PCR. For this purpose, total RNA had been isolated from long-term cultured macrophages that secreted large amounts of chitotriosidase activity. First strand cDNA synthesis was performed using SuperScript TM RNAse H, reverse transcriptase and oligo dT. After alkaline hydrolysis, the cDNA was precipitated with ethanol and used as template. PCR was performed using standard conditions. The DNA fragment obtained by RT-PCR was of the expected size (on the basis of homology with members of the chitinase family). The fragment was purified, treated with T4 DNA polymerase and cloned into the HindII site of the plasmid vector pUC19. Determination of its sequence using the dideoxynucleotide chain termination method revealed that the fragment was in complete accordance with the known N-terminal amino acid sequence of purified human chitotriosidase, allowing its use as a probe to identify a full length chitotriosidase cDNA.

DEPR:
The nucleotide sequence of the cDNA clone chi.50 shows an open reading frame starting with an ATG at position 13 and ending with a TGA codon at position 1410 (see FIG. 1). The open reading frame encodes a protein with a characteristic N-terminal ER signal peptide, immediately followed by the N-terminal sequence established for the chitotriosidase protein. The cDNA sequence does not indicate the presence of potential N-linked glycosylation sites, which is consistent with the absence of N-linked glycans in isolated chitotriosidase. The predicted protein, after removal of the signal sequence, has a length of 445 amino acids and a calculated molecular mass of 49 kDa. Metabolic labelling experiments with cultured macrophages revealed that these cells predominantly synthesize and secrete a chitotriosidase protein with apparent molecular mass of 50 kDa with polyacrylamide gel electrophoresis in the presence of sodium dodecylsulphate at reducing conditions. The predicted C-terminal part of 50 kDa human chitotriosidase is rich in serine residues of which theoretically some might be O-linked glycosylated. The occurrence of this type of glycans in 50 kDa human chitotriosidase has so far not been excluded or confirmed.

DEPR:
A search of the EMBL and GenBank databases revealed significant homology between the two human chitotriosidases and a group of chitinases and related proteins from different species. All the homologous proteins belong to the so called `chitinase protein family` (18, 19).

DEPR:
The predicted C-terminal part of 50 kDa human chitotriosidase shows only homology with two chitinases from Manduca sexta and Brugia malayi, respectively. In the case of the latter enzyme O-linked glycosylation has been reported (12).

DEPR:
The chitotriosidase produced by COS cells was analysed by immunotitration with a rabbit antiserum against human chitotriosidase. This antiserum is capable of inhibiting human chitotriosidase in its enzymatic activity. FIG. 5 shows that chitotriosidase is inactivated by the antiserum in an identical manner to isolated splenic chitotriosidase. This finding suggests that the enzymatic activity per amount of antigen is similar in the case of the two recombinant

chitotriosidases and the splenic enzyme.

DEPR:
Chitin azure (Sigma) suspended in citrate/phosphate buffer (pH 5.2) at a final concentration of 10 mg/ml was used to monitor chitinase activity. Chitin degradation was detected spectrophotometrically at 550 nm by determination of release of soluble azure (18). Chitinase from Serratia marcescans (Sigma) was used as control. When related to the hydrolysis of 4-methylumbelliferyl-chitotrioside, the **chitinase activity of human chitotriosidase** was comparable to that of the bacterial chitinase. See for example ref. 18.

DEPR:
No significant activity of **human chitotriosidase** towards a cell wall suspension of Micrococcus lysodeikticus was detectable, suggesting that the enzymes lack lysozyme activity.

DEPR:
To test whether **human chitotriosidase** can exert an anti-fungal action, a chitinous fungus (Mucor mucedo) was grown on plates (containing malt extract, peptone, glucose and agar) under a Cellophane membrane in order to keep the hyphae flat against the agar surface (see ref. 16). Individual sectors were cut out and mounted on microscope slides. Purified chitozyme 50 and chitozyme 39 were dialysed against 0.19 M sodium chloride. Samples of enzyme-containing solutions, and 0.15 M NaCl were pipetted on the hyphal tips. Microscopical analysis revealed that application of enzyme resulted in immediate cessation of hyphal growth, followed by a distorted morphological appearance. Applicant of saline had no effect. Negative effects on hyphal growth were detectable using chitozyme solutions with a concentration of enzyme as little as 0.005 mg/ml.

DEPR:
The proteins are: **human chitotriosidase**; a chitinase from the virus Autographa californica (GenBank L22850); a chitinase from the tobacco hornworm Manduca sexta (GenBank U02270); an endochitinase from the nematode Brugia malayi (GenBank M73689); a human oviductal glycoprotein (GenBank U09550); HCgp-39, a human glycoprotein produced by chondrocytes and synovial cells (GenBank M80927); YM-1, a secretory protein of activated mouse macrophages (Pir S27879); a chitinase from the fungus Aphanocladium album (SwissProt P32470); a chitinase from the filamentous fungus Trichoderma harzianum (GenBank L14614); chitinase A1 from the prokaryote Bacillus circulans (SwissProt P20533); and a class V chitinase form the plant Nicotiana tabacum (GenBank X77110). Residues identical to chitotriosidase are indicated by the inverted characters. The proteins HCgp-39 and YM-1 are supposed to be not chitinolytic.

DEPR:
Preparations containing either purified 39 kDa splenic chitotriosidase (), or 50 kDa chitozyme produced by COS cells transfected with chi.50 cDNA (), or 39 kDa chitozyme produced by COS cells transfected with chi.39 cDNA were incubated for 1 hour at room temperature in phosphate buffered saline with different amounts of rabbit (anti-**human splenic chitotriosidase**) antiserum.

DEPC:
Cloning and composition of cDNAs encoding **human chitotriosidases**

DEPC:
Recombinant production of **human chitotriosidases**

DEPU:
18. Renkema, G. E., Boot. R. G., Muijsers, A. O., Donker Koopman, W. E., Aerts, J. M. F. G. (1995), J. Biol. Chem. 270, 2196-2202. Purification and characterization of **human chitotriosidase,** a novel member of the chitinase family of proteins.

CLPR:
1. A substantially isolated or purified **chitinase, said chitinase being a human chitinase or being a modified form of said human chitinase** having a substantially similar chitin-hydrolyzing activity which comprises an amino acid sequence selected from the group consisting of an amino acid sequence depicted

in SEQ. ID NOS: 4 and 6.

CLPR:
15. An isolated nucleic acid comprising a nucleotide sequence encoding a **human chitinase** wherein the nucleotide sequence comprises the nucleotide base sequence depicted in SEQ ID NO: 3 or SEQ ID NO: 5.

CLPR:
18. An antibody that is capable of specifically binding to an epitope of the human chitinase of claim 1.

CLPR:
23. A diagnostic kit comprising the **human chitinase** of claim 1 and a component for detecting an antigen or an antibody.

CLPR:
24. A method of decomposing chitin comprising contacting said chitin with the human chitinase of claim 1 under chitin-hydrolyzing conditions.

USPT

US-CL-CURRENT: 424/535,424/548 ,514/2 ,514/21 ,514/825 ,530/350 ,530/395

US-PAT-NO: 5843449
DOCUMENT-IDENTIFIER: US 5843449 A
TITLE: Proteins and novel peptides derived from autoantigen for use in immunotherapy of autoimmune diseases
DATE-ISSUED: December 1, 1998
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Boots; Anna Maria	Megen	N/A	N/A	NLX
Helena	Oss	N/A	N/A	NLX
Verheijden; Gijsbertus Franciscus Maria	Oss	N/A	N/A	NLX
Bos; Ebo Sybren				

US-CL-CURRENT: 424/185.1,424/535 ,424/548 ,514/2 ,514/21 ,514/825 ,530/350 ,530/395
ABSTRACT:
The present invention relates to novel peptides derived from the autoantigen HC gp-39, said peptides comprising at least one of the amino acid sequences FGRSFTILAS (SEQ ID No. 1), FTLASSETG (SEQ ID No. 2), YDDQESVKS (SEQ ID No. 3) and FSKIASNTQ (SEQ ID No. 4). The peptides resemble MHC Class II restricted T-cell epitopes present on the autoantigen HC gp-39 in articular cartilage. HC gp-39, proteins comprising an amino acid sequence which exhibits at least 50% homology with the amino acid sequence YKLVCYYTSWSQYREGDGSCFPDALDRFLCTHIIYSFANISND (SEQ ID No: 10) and said peptides can be used in antigen-specific treatment of articular cartilage destruction in autoimmune diseases in mammals to induce systemic tolerance of the immune system. The autoantigen HC gp-39, proteins comprising an amino acid sequence which exhibits at least 50% homology with the amino acid sequence YKLVCYYTSWSQYREGDGSCFPDALDRFLCTHIIYSFANISND (SEQ ID NO: 10) and said peptides are also suitable to induce arthritis in animals, preferably mice. The invention furthermore relates to pharmaceutical compositions comprising said autoantigen and/or said peptides, a diagnostic method for the detection of autoreactive T cells in a test sample and test kits to be used in said method.
8 Claims, 5 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 5

BSPR:
Suitable arthritogenic proteins according to the invention are for example pig heparine-binding 38 kDa protein, bovine, 39 kDa whey protein, murine breast regressing 39 kDa protein (brp39), human oviduct-specific glycoprotein, murine oviduct-specific glycoprotein, hamster oviduct-specific glycoprotein, bovine oviduct-specific glycoprotein, human chitotriosidase precursor protein and murine secretory protein YM-1 precursor. The arthritogenic proteins according to the invention are very suitable for inducing systemic tolerance of the immune system to homologous autoantigens and can be used to delay and/or suppress arthritic development in mammals.

BSPR:
Proteins of which the amino acid sequence exhibits at least 50% homology with the amino acid sequence of HC gp-39, more in particular with the amino acid sequence YKLVCYYTSWSQYREGDGSCFPPDALDRFLCTHIIYSFANISND (SEQ ID NO: 10) have been described. The identification of pig heparine-binding 38 kDa protein is described in Shackelton et al. (1995), J. Biol. Chem. Vol. 270, No. 22, 13076-13083, however no function of the protein was identified. The isolation and characterization of bovine 39 kDa whey protein is described in J. J. Rejman et al. (1988), Biochem. Biophys. Res. Comm. Vol. 150, No. 1, 329-334. Murine breast regressing 39 kDa protein (brp39) is described in Morrison and Leder, 1994, Oncogene 9, 3417. Cloning of the cDNA encoding human oviduct-specific glycoprotein and the corresponding amino acid sequence is described in Arias et al. (1994), Biology of Reproduction 51, 685-694. Other mammalian oviduct-specific glycoproteins such as murine- and hamster oviduct-specific glycoprotein are disclosed in JP-A-07107979, Kinosei Peptide

Kenkyusho KK. The purification and molecular cloning of the bovine oviduct-specific glycoprotein is described in Y. Sendai et al, 1994, Biol. of Reprod. 50, 927-934. Human chitotriosidase precursor protein is secreted by activated human marcophages and the cloning of the corresponding cDNA and amino acid sequence is described in Boot et al. (1995), J. Biol. Chem. Vol. 270, No. 44, 26252-26256. The amino acid sequence of murine secretory protein YM-1 precursor was submitted to EMBL Data Library, June 1992, Accession No. M94584 by Chang et al., unpublished. None of the afore-mentioned publications however hint or suggest towards the arthritogenic nature of the proteins according to the invention nor to the fact that these proteins can be used as a medical substance in a therapy to induce specific T-cell tolerance to HC gp-39 in mammals, more specifically man, suffering from T-cell mediated cartilage destruction, such as for example arthritis, more specifically rheumatoid arthritis.

BSPR:
Very suitable proteins to be used in a pharmaceutical composition according to the invention are for example pig heparine-binding 38 kDa protein, bovine 39 kDa whey protein, murine breast regressing 39 kDa protein (brp39), murine oviduct-specific glycoprotein, hamster oviduct-specific glycoprotein, bovine oviduct-specific glycoprotein, human oviduct-specific glycoprotein, human chitotriosidase precursos protein and murine secretory protein YM-1 precursor.

CLPR:
1. A method for treating mammals suffering from a T-cell mediated cartilage destruction disease, comprising administering a T-cell specific tolerance inducing amount of a composition comprising one or more proteins selected from the group consisting of pig heparin binding 38 kDa protein, bovine 39 kDa whey protein, murine breast regressing 39 kDa protein (brp39), human oviduct-specific glycoprotein, murine oviduct-specific glycoprotein, hamster oviduct-specific glycoprotein, bovine oviduct-specific glycoprotein, human chitotriosidase precursor protein, and murine secretory protein YM-1 precursor, and fragments of the foregoing proteins that will induce said T-cell specific tolerance, together with a pharmaceutically acceptable carrier.

CLPR:
5. A method of inducing arthritis in an animal to provide an animal model for the study of arthritis, comprising administering to said animal an arthritis inducing amount of a composition comprising one or more proteins selected from the group consisting of pig heparin binding 38 kDa protein, bovine 39 kDa whey protein, murine breast regressing 39 kDa protein, (brp39), human oviduct-specific glycoprotein, murine oviduct-specific glycoprotein, hamster oviduct-specific glycoprotein, bovine oviduct-specific glycoprotein, human chitotriosidase precursor protein, and murine secretory protein YM-1 precursor, and fragments of the foregoing proteins that will induce arthritis in an animal, together with a pharmaceutically acceptable carrier.

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  * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 08:18:42 ON 16 NOV 2000

=> fil .bec

COST IN U.S. DOLLARS                                    SINCE FILE      TOTAL
                                                           ENTRY    SESSION
FULL ESTIMATED COST                                         0.15       0.15

FILES 'MEDLINE, SCISEARCH, LIFESCI, BIOTECHDS, BIOSIS, EMBASE, HCAPLUS, NTIS,
       ESBIOBASE, BIOTECHNO' ENTERED AT 08:19:24 ON 16 NOV 2000
ALL COPYRIGHTS AND RESTRICTIONS APPLY.  SEE HELP USAGETERMS FOR DETAILS.

10 FILES IN THE FILE LIST

=> s chitinase# or chitotriosidase#

FILE 'MEDLINE'
          1094 CHITINASE#
            34 CHITOTRIOSIDASE#
L1          1115 CHITINASE# OR CHITOTRIOSIDASE#

FILE 'SCISEARCH'
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            50 CHITOTRIOSIDASE#
L2          2409 CHITINASE# OR CHITOTRIOSIDASE#

FILE 'LIFESCI'
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             5 CHITOTRIOSIDASE#
L3          1131 CHITINASE# OR CHITOTRIOSIDASE#

FILE 'BIOTECHDS'
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             1 CHITOTRIOSIDASE#
L4          746 CHITINASE# OR CHITOTRIOSIDASE#

FILE 'BIOSIS'
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L5          2923 CHITINASE# OR CHITOTRIOSIDASE#

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L7          3326 CHITINASE# OR CHITOTRIOSIDASE#

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FILE 'ESBIOBASE'
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L10        947 CHITINASE# OR CHITOTRIOSIDASE#

TOTAL FOR ALL FILES
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L12         27 L1 (5A)HUMAN

FILE 'SCISEARCH'
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L13         31 L2 (5A)HUMAN

FILE 'LIFESCI'
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L14         15 L3 (5A)HUMAN

FILE 'BIOTECHDS'
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L15          9 L4 (5A)HUMAN

FILE 'BIOSIS'
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L16         35 L5 (5A)HUMAN

FILE 'EMBASE'
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L17         26 L6 (5A)HUMAN

FILE 'HCAPLUS'
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L18         45 L7 (5A)HUMAN

FILE 'NTIS'
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L19          0 L8 (5A)HUMAN

FILE 'ESBIOBASE'
        242589 HUMAN
L20         21 L9 (5A)HUMAN

FILE 'BIOTECHNO'
        557159 HUMAN
L21         20 L10(5A)HUMAN

TOTAL FOR ALL FILES
L22        229 L11(5A) HUMAN

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L23         0 L12 AND PY=<1996

FILE 'SCISEARCH'
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L24         0 L13 AND PY=<1996

FILE 'LIFESCI'
        11326 PY=<1996
L25         0 L14 AND PY=<1996

FILE 'BIOTECHDS'
          104 PY=<1996
                (PY=<1996)
L26         0 L15 AND PY=<1996

FILE 'BIOSIS'
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L27         0 L16 AND PY=<1996

FILE 'EMBASE'
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L28         0 L17 AND PY=<1996

FILE 'HCAPLUS'
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L29         3 L18 AND PY=<1996

FILE 'NTIS'
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L30         0 L19 AND PY=<1996

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L32         7 L21 AND PY=<1996

TOTAL FOR ALL FILES
L33        10 L22 AND PY=<1996

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PROCESSING COMPLETED FOR L33
L34         10 DUP REM L33 (0 DUPLICATES REMOVED)

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L34   ANSWER 1 OF 10  BIOTECHNO  COPYRIGHT 2000 Elsevier Science B.V.
TI    Molecular cloning of a third chitinase gene (CHT1) from Candida albicans
SO    Yeast, (1996), 12/5 (501-504)
      CODEN: YESTE3  ISSN: 0749-503X
AU    McCreath K.J.; Specht C.A.; Liu Y.; Robbins P.W.
AN    1996:26157855   BIOTECHNO

L34  ANSWER 2 OF 10  HCAPLUS  COPYRIGHT 2000 ACS
TI   Chitinase levels in guinea pig blood are increased in experimentally
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induced aspergillosis
SO Chitin Enzymol., Proc. Int. Symp., 2nd (**1996**), 21-36. Editor(s): Muzzarelli, Riccardo A. A. Publisher: Atec Edizioni, Grottammare, Italy. CODEN: 65LZA3
AU Overdijk, Bernard; Van Steijn, Ge J.; Odds, Frank C.
AN 1998:49330 HCAPLUS
DN 128:87217

L34 ANSWER 3 OF 10 HCAPLUS COPYRIGHT 2000 ACS
TI Expression of chitinase activity during monocyte differentiation
SO Chitin Enzymol., Proc. Int. Symp., 2nd (**1996**), 11-20. Editor(s): Muzzarelli, Riccardo A. A. Publisher: Atec Edizioni, Grottammare, Italy. CODEN: 65LZA3
AU Escott, Gemma M.; Walters, Christina E.; Ingham, Eileen; Adams, David J.
AN 1998:49325 HCAPLUS
DN 128:113353

L34 ANSWER 4 OF 10 HCAPLUS COPYRIGHT 2000 ACS
TI **Chitotriosidase**: a **human** macrophage **chitinase** that is a marker for Gaucher disease manifestation
SO Chitin Enzymol., Proc. Int. Symp., 2nd (**1996**), 3-10. Editor(s): Muzzarelli, Riccardo A. A. Publisher: Atec Edizioni, Grottammare, Italy. CODEN: 65LZA3
AU Aerts, J. M. F. G.; Boot, R. G.; Renkema, G. H.; van Weely, S.; Hollak, C. E. M.; Donker-Koopman, W.; Strijland, A.; Verhoek, M.
AN 1998:49322 HCAPLUS
DN 128:73525

L34 ANSWER 5 OF 10 BIOTECHNO COPYRIGHT 2000 Elsevier Science B.V.
TI Cloning of a cDNA encoding **chitotriosidase**, a **human chitinase** produced by macrophages
SO Journal of Biological Chemistry, (**1995**), 270/44 (26252-26256) CODEN: JBCHA3 ISSN: 0021-9258
AU Boot R.G.; Renkema G.H.; Strijland A.; Van Zonneveld A.J.; Aerts J.M.F.G.
AN 1995:25333011 BIOTECHNO

L34 ANSWER 6 OF 10 BIOTECHNO COPYRIGHT 2000 Elsevier Science B.V.
TI **Chitinase** activity in **human** serum and leukocytes
SO Infection and Immunity, (**1995**), 63/12 (4770-4773) CODEN: INFIBR ISSN: 0019-9567
AU Escott G.M.; Adams D.J.
AN 1995:25347656 BIOTECHNO

L34 ANSWER 7 OF 10 BIOTECHNO COPYRIGHT 2000 Elsevier Science B.V.
TI Purification and characterization of **human chitotriosidase**, a novel member of the chitinase family of proteins
SO Journal of Biological Chemistry, (**1995**), 270/5 (2198-2202) CODEN: JBCHA3 ISSN: 0021-9258
AU Renkema G.H.; Boot R.G.; Muijsers A.O.; Donker-Koopman W.E.; Aerts J.M.F.G.
AN 1995:25039681 BIOTECHNO

L34 ANSWER 8 OF 10 BIOTECHNO COPYRIGHT 2000 Elsevier Science B.V.
TI Differential recognition of microfilarial chitinase, a transmission-blocking vaccine candidate antigen, by sera from patients with Brugian and Bancroftian filariasis
SO American Journal of Tropical Medicine and Hygiene, (**1995**), 53/3

(289-294)
CODEN: AJTHAB ISSN: 0002-9637
AU Dissanayake S.; Perler F.B.; Xu M.; Southworth M.W.; Yee C.K.; Wang S.; Dreyer G.; Watawana L.; Kurniawan L.; Fuhrman J.A.; Piessens W.F.
AN 1995:25303493 BIOTECHNO

L34 ANSWER 9 OF 10 BIOTECHNO COPYRIGHT 2000 Elsevier Science B.V.
TI Cloning and expression in Escherichia coli of the nahA gene from Porphyromonas gingivalis indicates that .beta.-N-acetylhexosaminidase is an outer-membrane-associated lipoprotein
SO Microbiology, (**1994**), 140/12 (3399-3406)
CODEN: MROBEO ISSN: 1350-0872
AU Lovatt A.; Roberts I.S.
AN 1994:25020626 BIOTECHNO

L34 ANSWER 10 OF 10 BIOTECHNO COPYRIGHT 2000 Elsevier Science B.V.
TI The coccidioidal complement fixation and immunodiffusion-complement fixation antigen is a chitinase
SO Infection and Immunity, (**1992**), 60/7 (2588-2592)
CODEN: INFIBR ISSN: 0019-9567
AU Johnson S.M.; Pappagianis D.
AN 1992:22219606 BIOTECHNO

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